Molecular Genetics and Metabolism 119 (2016) 160–167

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism

journal homepage: www.elsevier.com/locate/ymgme

Diagnosis of neuronal ceroid lipofuscinosis type 2 (CLN2 disease): Expert recommendations for early detection and laboratory diagnosis

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ARTICLE INFO

Article history:
Received 26 May 2016
Received in revised form 23 July 2016
Accepted 24 July 2016
Available online 25 July 2016

Keywords:
Neuronal ceroid lipofuscinosis
Laboratory diagnosis
Lysosomal storage disorder
Expert recommendations
Neurodegeneration
Genetic cause of epilepsy

ABSTRACT

Neuronal ceroid lipofuscinoses (NCLs) are a heterogeneous group of lysosomal storage disorders. NCLs include the rare autosomal recessive neurodegenerative disorder neuronal ceroid lipofuscinosis type 2 (CLN2) disease, caused by mutations in the tripeptidyl peptidase 1 (*TPP1*)/*CLN2* gene and the resulting TPP1 enzyme deficiency. CLN2 disease most commonly presents with seizures and/or ataxia in the late-infantile period (ages 2–4), often in combination with a history of language delay, followed by progressive childhood dementia, motor and visual deterioration, and early death. Atypical phenotypes are characterized by later onset and, in some instances, longer life expectancies. Early diagnosis is important to optimize clinical care and improve outcomes; however, currently, delays in diagnosis are common due to low disease awareness, nonspecific clinical presentation, and limited access to diagnostic testing in some regions. In May 2015, international experts met to recommend best laboratory practices for early diagnosis of CLN2 disease. When clinical signs suggest an NCL, TPP1 enzyme activity should be among the first tests performed (together with the palmitoyl-protein thioesterase enzyme activity assay to rule out CLN1 disease). However, reaching an initial suspicion of an NCL or CLN2 disease can be challenging; thus, use of an epilepsy gene panel for investigation of unexplained seizures in the late-infantile/childhood ages is encouraged. To confirm clinical suspicion of CLN2 disease, the recommended gold standard for laboratory diagnosis is demonstration of deficient TPP1 enzyme activity (in leukocytes, fibroblasts, or dried blood spots) and the identification of causative mutations in each allele of the *TPP1*/*CLN2* gene. When it is not possible to perform both analyses, either demonstration of a) deficient TPP1 enzyme activity in leukocytes or fibroblasts, or b) detection of two pathogenic mutations in *trans* is diagnostic for CLN2 disease.

1. Introduction

Neuronal ceroid lipofuscinosis type 2 (CLN2) disease (OMIM 204500) is a rare autosomal recessive lysosomal storage disorder that results from deficient activity of the lysosomal exopeptidase tripeptidyl peptidase 1 (TPP1) enzyme (EC 3.4.14.9) caused by mutations in the

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http://dx.doi.org/10.1016/j.ymgme.2016.07.011

TPP1/*CLN2* gene (GenBank accession no. NM_000391.3) [1]. As in other neuronal ceroid lipofuscinosis (NCL) disorders (Table 1), CLN2 disease leads to intralysosomal accumulation of autofluorescent storage materials and neuronal loss.

The *in vivo* substrate(s) of TPP1 are not known, and the molecular pathology of TPP1 enzyme deficiency is poorly understood [2]. CLN2 disease incidence estimates range from 0.22 to 9.0 per 100,000 live births, but it is possible that it is under-recognized [2–4].

The classic phenotype of CLN2 disease is the most common form of NCL with late-infantile onset (roughly defined as ages 2–4 years) and generally manifests with new-onset seizures and/or ataxia, typically in combination with a history of early language delay [5]. Classic phenotype disease progression is rapid, leading to the loss of acquired developmental milestones, new or worsening ataxia, movement disorders (myoclonus, dystonia, and chorea), progressive dementia, and eventual loss of vision [3,5–9]. A majority of those diagnosed with CLN2 disease die prematurely after becoming bedridden and blind. The more rare atypical phenotypes are characterized by varied ages of initial presentation and/or longer life expectancy [5,10–12]. An example of atypical CLN2 disease is spinocerebellar ataxia autosomal recessive 7 (SCAR7; OMIM 609270), which was initially described as a distinct disorder but is in fact caused by deficient TPP1 enzyme activity; individuals with SCAR7 develop ataxia and cerebellar atrophy but do not develop seizures or loss of vision [12].

In May 2015, 13 international laboratory and clinical NCL experts met both to develop a CLN2 disease–specific diagnostic algorithm (Fig. 1) and to define the gold standard diagnostic laboratory tests to support an early and accurate diagnosis of CLN2 disease.

2. Clinical suspicion and paths to diagnosis of CLN2 disease

Developing a specific suspicion of CLN2 disease through differential diagnosis is often a protracted process, due in part to lack of pathognomonic signs at onset. Based on the expert meeting discussions, three general paths lead to a laboratory diagnosis of CLN2 disease, depending on degree of CLN2 disease suspicion (Fig. 1).

2.1. High suspicion of CLN2 disease

CLN2 disease is rarely suspected or diagnosed at initial presentation to the clinic unless there is already a known, affected family member. Because CLN2 disease is one of the most common of NCL disorders [13], children who ultimately present to an NCL specialist with ataxia, worsening unprovoked seizures, history of language delay, and/or are at a developmental stand-still will follow a clear, direct path specific for a high suspicion of classic CLN2 disease (Fig. 1, center) [5]. Typically, diagnosis only occurs after a series of misdiagnoses, as the early disease course is clinically similar to many other seizure and/or metabolic disorders.

An EEG is often the first clinical test performed irrespective of the level of specific clinical suspicion (Fig. 1). In cases of CLN2 disease, electroencephalograms (EEGs) may detect irregular activity, a slowing of background activity, and epileptiform abnormalities in posterior regions. Visual evoked potentials reveal an increase of latency; optical coherence tomography may detect ocular abnormalities; and electroretinograms may be diminished [3,5,8,14,15]. Ocular abnormalities and vision defects can be subtle initially but increase in prominence with disease progression [5,8,15].

Table 1
The neuronal ceroid lipofuscinosis disorders.
Adapted from the DEM-CHILD algorithm, http://www.dem-child.eu/index.php/ncl-diagnostic-algorithm.html, and Schulz et al. 2013 [14].

Age and clinical presentation	Genes	Protein products, in bold if enzyme assay widely available	Classical clinical presentation by age group
Newborns (through infantile)			
• Epilepsy	*CLN1*	**PPT1** (lysosomal lipid hydrolase)	Classical
• Microcephaly	*CLN10*	**CtsD** (lysosomal peptidase)	Classical
	CLN14	KCTD7 (unknown function; homology to potassium channel tetramerization domain)	Classical
Young children (late infantile)			
• Developmental delay or regression	*CLN1*	**PPT1** (lysosomal lipid hydrolase)	
• Newly occurring epilepsy of unknown cause	*CLN2*	**TPP1** (lysosomal exopeptidase)	Classical
	CLN5	(Soluble lysosomal protein)	Classical
	CLN6	(Transmembrane protein)	Classical
	CLN7	MFSD8 (transmembrane protein)	Classical
	CLN8	(Transmembrane protein)	Classical
	CLN10	**CtsD** (lysosomal peptidase)	
School-aged children (juvenile)			
• Vision loss	*CLN1*	**PPT1** (lysosomal lipid hydrolase)	
• Dementia	*CLN2*	**TPP1** (lysosomal exopeptidase)	
• Epilepsy	*CLN3*	(Transmembrane protein)	Classical
	CLN5	(Soluble lysosomal protein)	
	CLN6	(Transmembrane protein)	
	CLN7	MFSD8 (transmembrane protein)	
	CLN8	(Transmembrane protein)	
	CLN10	**CtsD** (lysosomal peptidase)	
	CLN12	ATP13A2 (ATPase)	Classical
Young adults			
• Nonspecific mental, motor, or behavioral abnormalities	*CLN1*	**PPT1** (lysosomal lipid hydrolase)	
	CLN2	**TPP1** (lysosomal exopeptidase)	
	CLN4 (autosomal dominant)	DNAJC5 (HSP40/DNAJ protein)	Classical
	CLN5	(Soluble lysosomal protein)	
	CLN6	(Transmembrane protein)	
	CLN10	**CtsD** (lysosomal peptidase)	
	CLN11	GRN (progranulin)	Classical
	CLN13	CtsF (lysosomal proteinase)	Classical

CLN, neuronal ceroid lipofuscinosis; Cts, cathepsin; GRN, granulin; HSP40, heat shock protein 40; KCTD7, potassium channel tetramerization domain containing 7; MFSD8, major facilitator superfamily domain containing 8; PPT1, palmitoyl-protein thioesterase 1; TPP1, tripeptidyl peptidase 1.